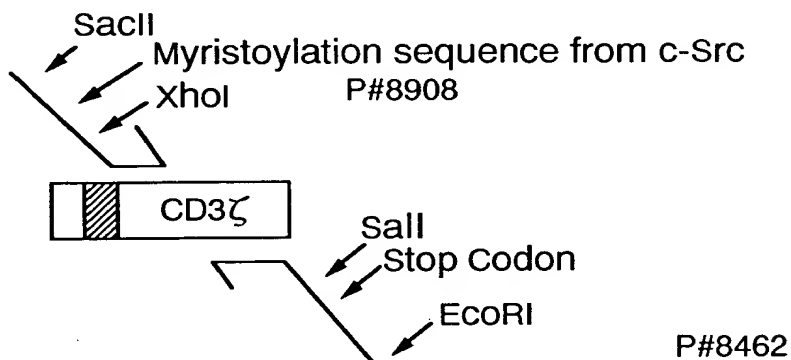


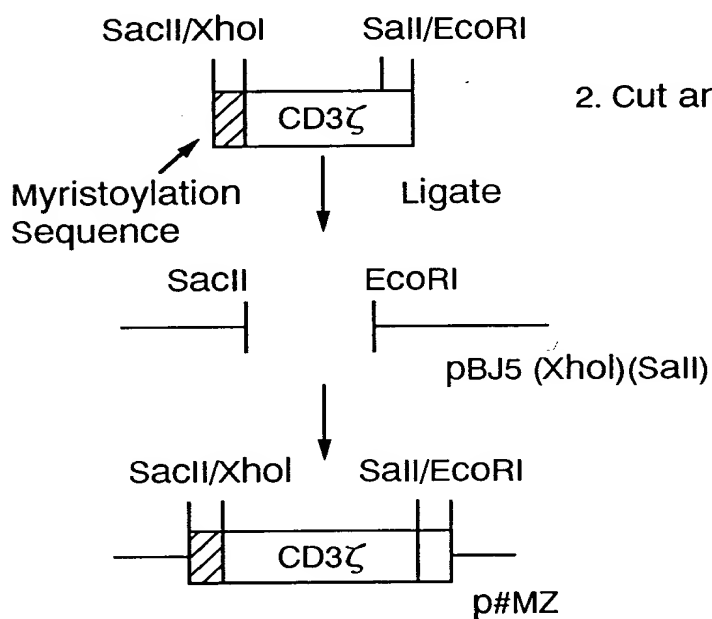
FIG. 1

Construction of intracellular signalling chimera:

1. PCR myristoylated CD3 ζ



2. Cut and clone PCR fragment



*The MZE series contains a 9aa HA epitope at the 3' end.

3. SEQUENCE insert

4. Cut at XhoI or SalI and add FKBP domains

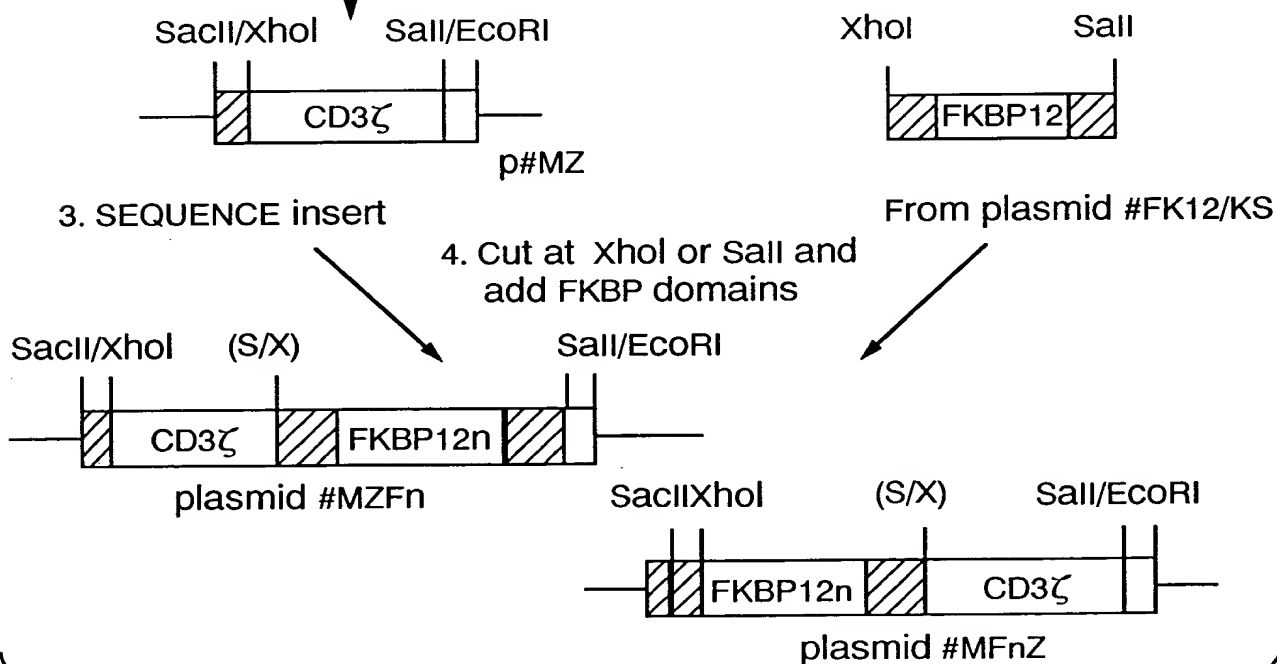
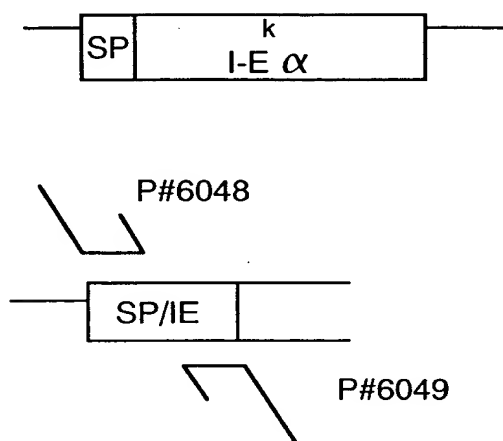


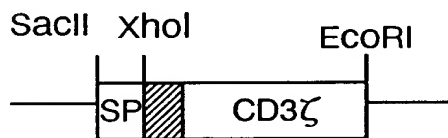
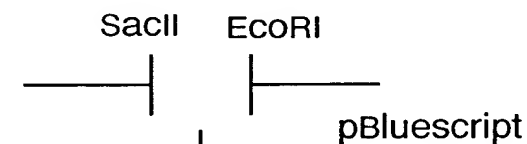
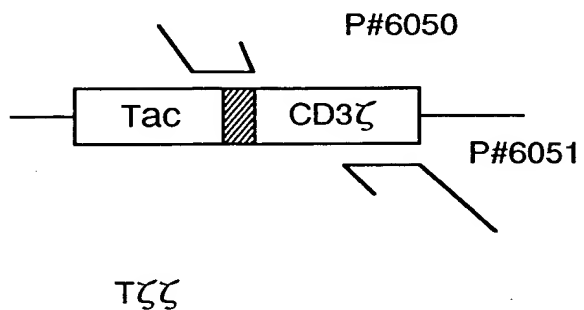
FIG. 2

Construction of extracellular signaling chimera:

1. PCT murine signal peptide



2. PCT CD3 trans-membrane and cytoplasmic domains



plasmid #SPZ/KS
SEQUENCE insert*

Cut XhoI

FIG. 3A

3. PCR FKBP12

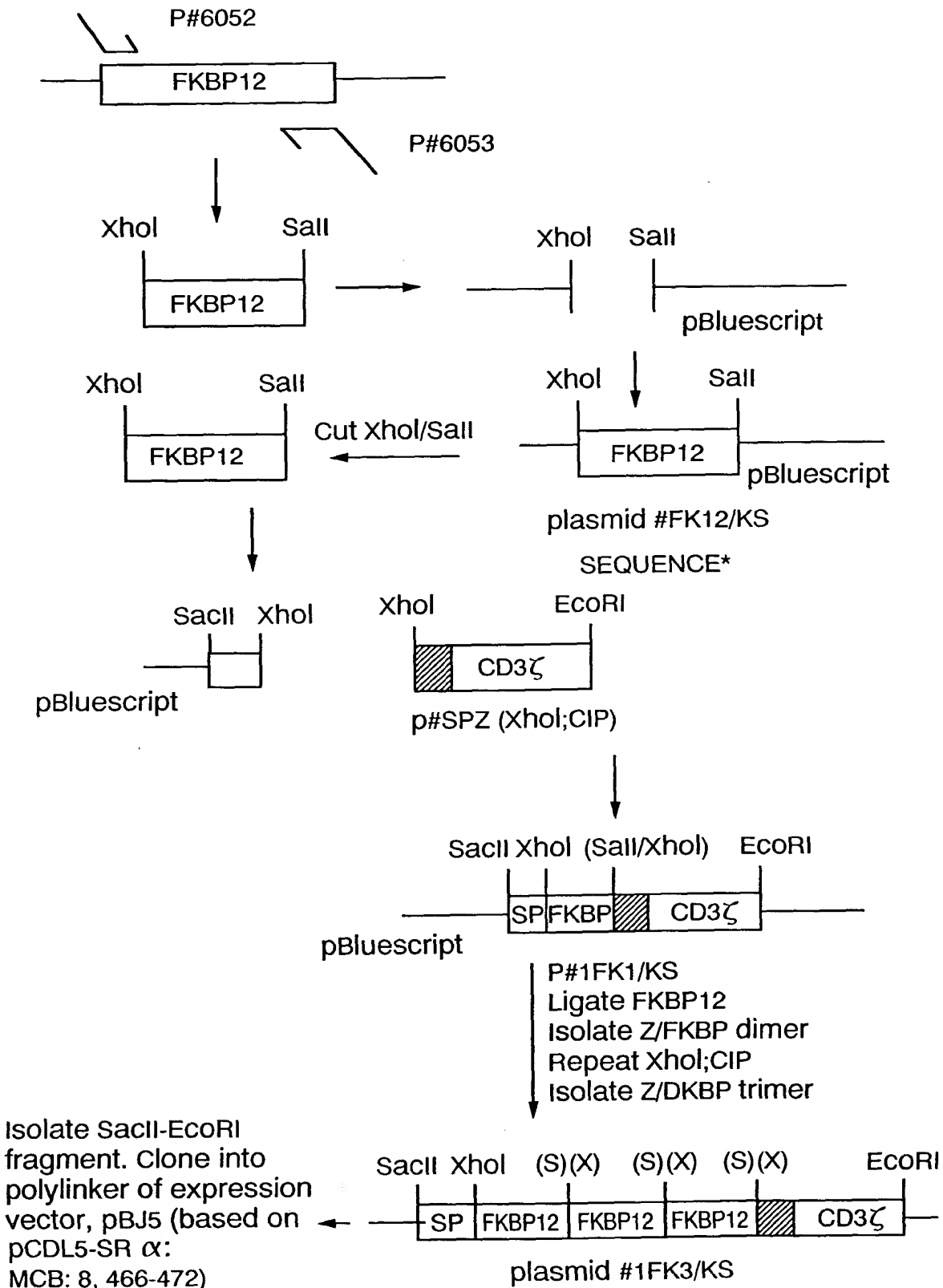


FIG. 3B

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CYCC



EPITOPE

7851: 5' - Sall TCGACTGCGTAGTCTGGTACGTCGTACGGATAC - 3'

EPITOPE: 5SEP, 3XEP

8922: 5' - TCGACTATCCGTACGACGTACCAGACTACGCAC - 3' SalI

8923: 5' - TCGAGTGCGTAGTCTGGTACGTCGTACGGATAG - 3' XhoI



Myristoylation from c-src 5SMXZ

8908: 5' -CGACACCGCGGCCACCATGGGGAGTAGCAAGAGCAAGCCT
KOZAK M G S S K S K P

8912: 5' -CGACACTCGAGGAGCTCTGTGACGATG-3'

xhoI homology

E L C D D

FIG. 4B

[illegible]

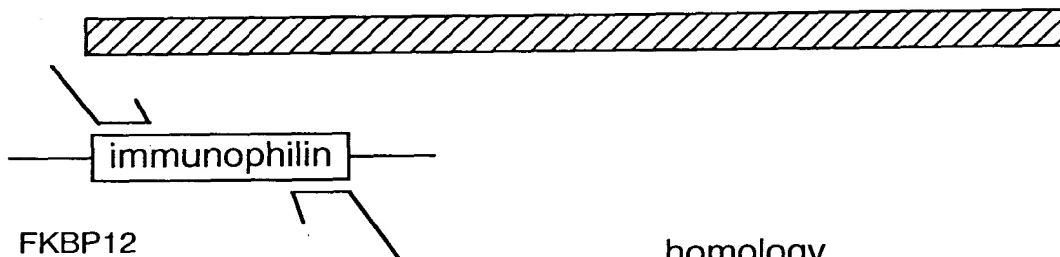
7/36

Asp-Lys #4 XhoI homology
8061: 5' -CGACACTCGAGCTCTGCTACTTGCTAAAGGGAATCCTCTTC-3'
 E L C Y L L K G I L F

*GATtoAAG

#4 Extension XhoI homology
8907: 5' -CGACACTCGAGCTGCTGGATCCGAAGCTCTGCTACTTGCTAAAG-3'
 E L L D P K L C Y L L K

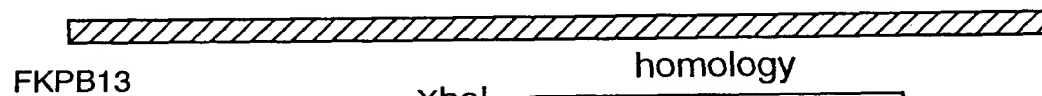
TAC-Tm #3 XhoI homology
7220: 5' -CGACACTCGAGACAACAGAGTACCAGGTAGC-3'
 E T T E Y Q V



FKBP12

6052: XhoI homology
5' -CGACACTCGAGGGCGTGCAGGTGGAGAC-3'
 E G V Q V E

6053: Sall homology
5' -CGACAGTCGACTTCCAGTTTTAGAAC-3'
 V E L K L L



FKPB13 XhoI homology
8460: 5' -TCGACACTCGAGACGGGGGCGGAGGGC-3'
 E T G A E G

8461: Sall homology
5' -CCGACAGTCGACCTCTATTTTGAGCAGC-3'
 V E I



FIG. 4C

8/36

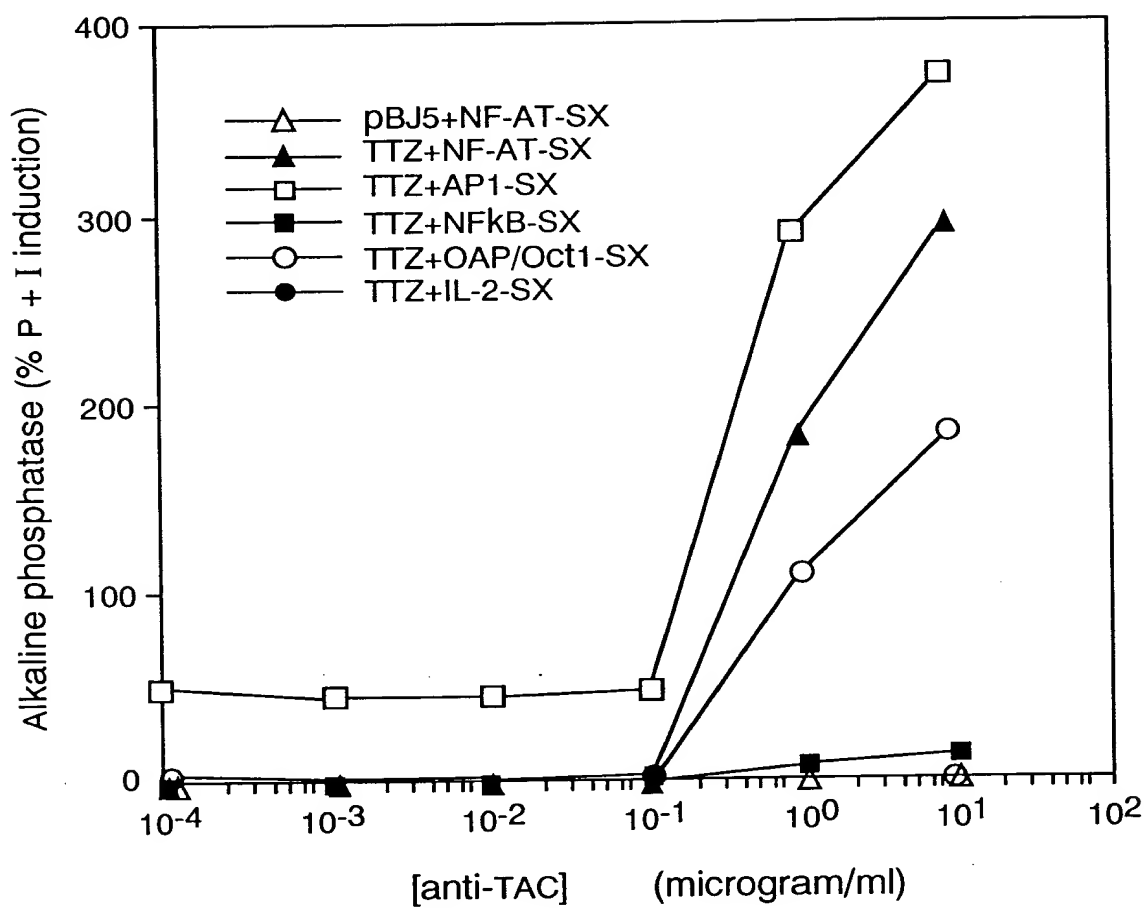


FIG. 5

9/36

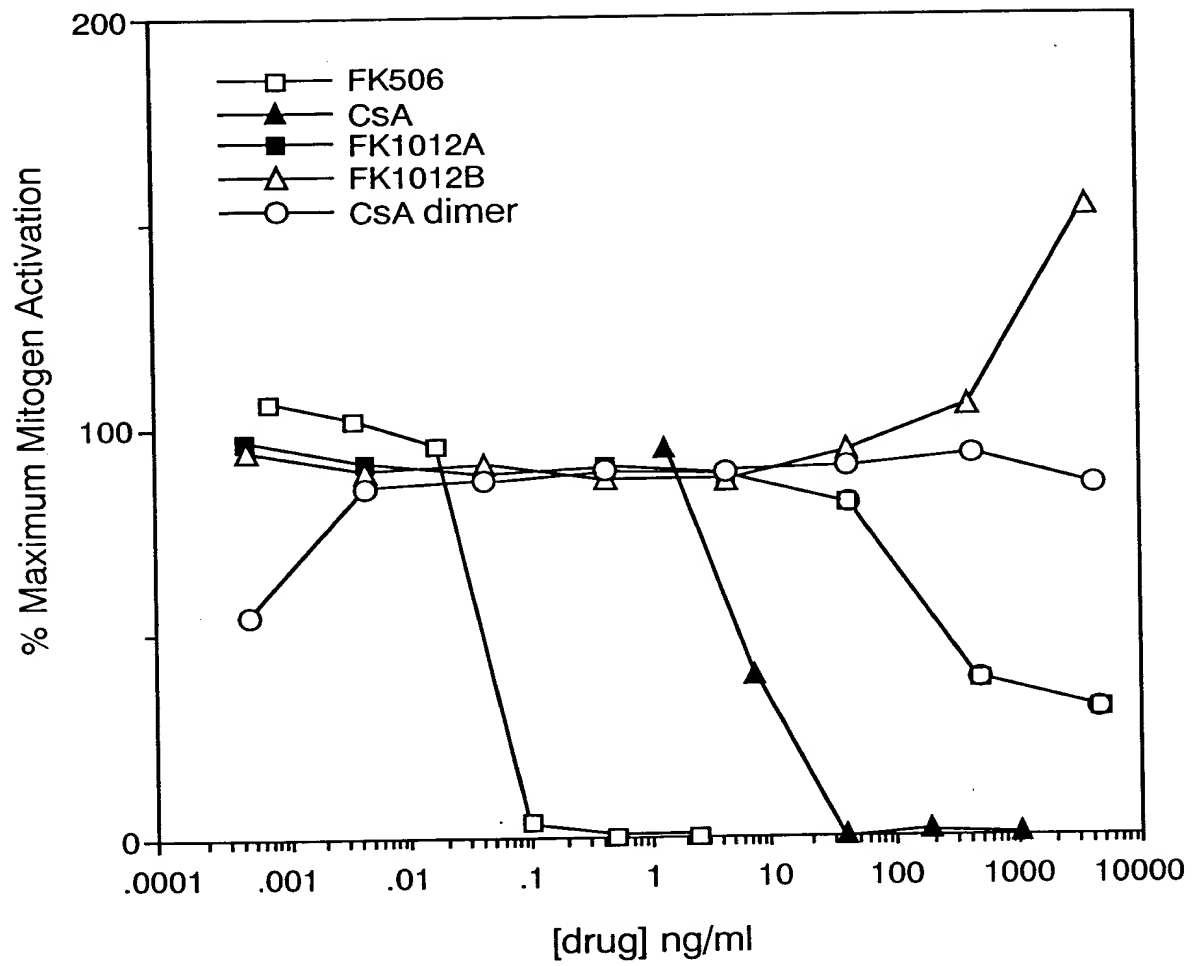
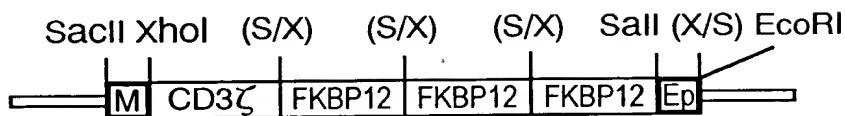
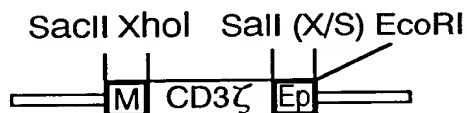


FIG. 6A

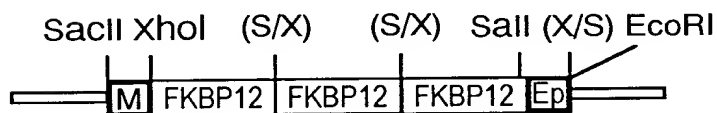


MZF3E

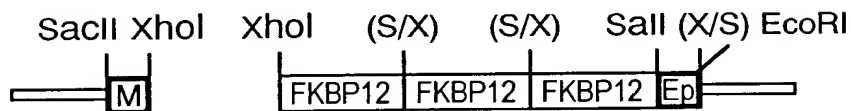


MZE

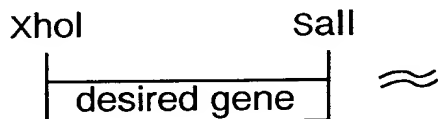
Cut XhoI/SalI; CIP; + FKBP12X3



MF3E



+



1. Cytoplasmic moiety of surface receptor
2. Tyrosine Kinase
3. Transcription Factor
4. Others

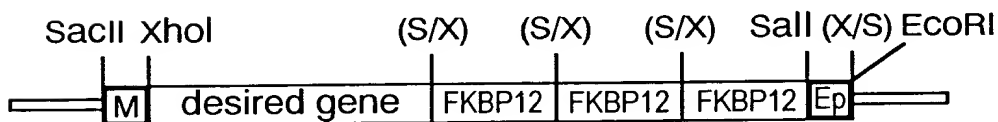


FIG. 6B

11/36

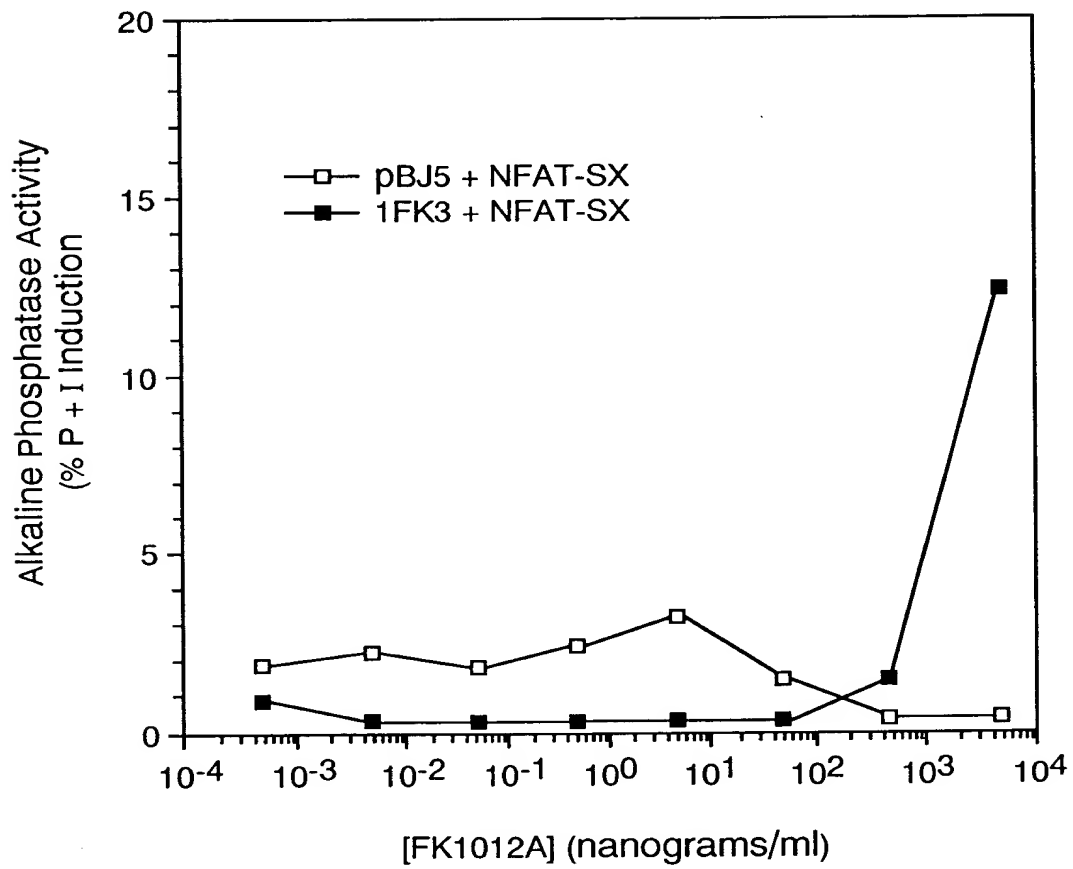


FIG. 7

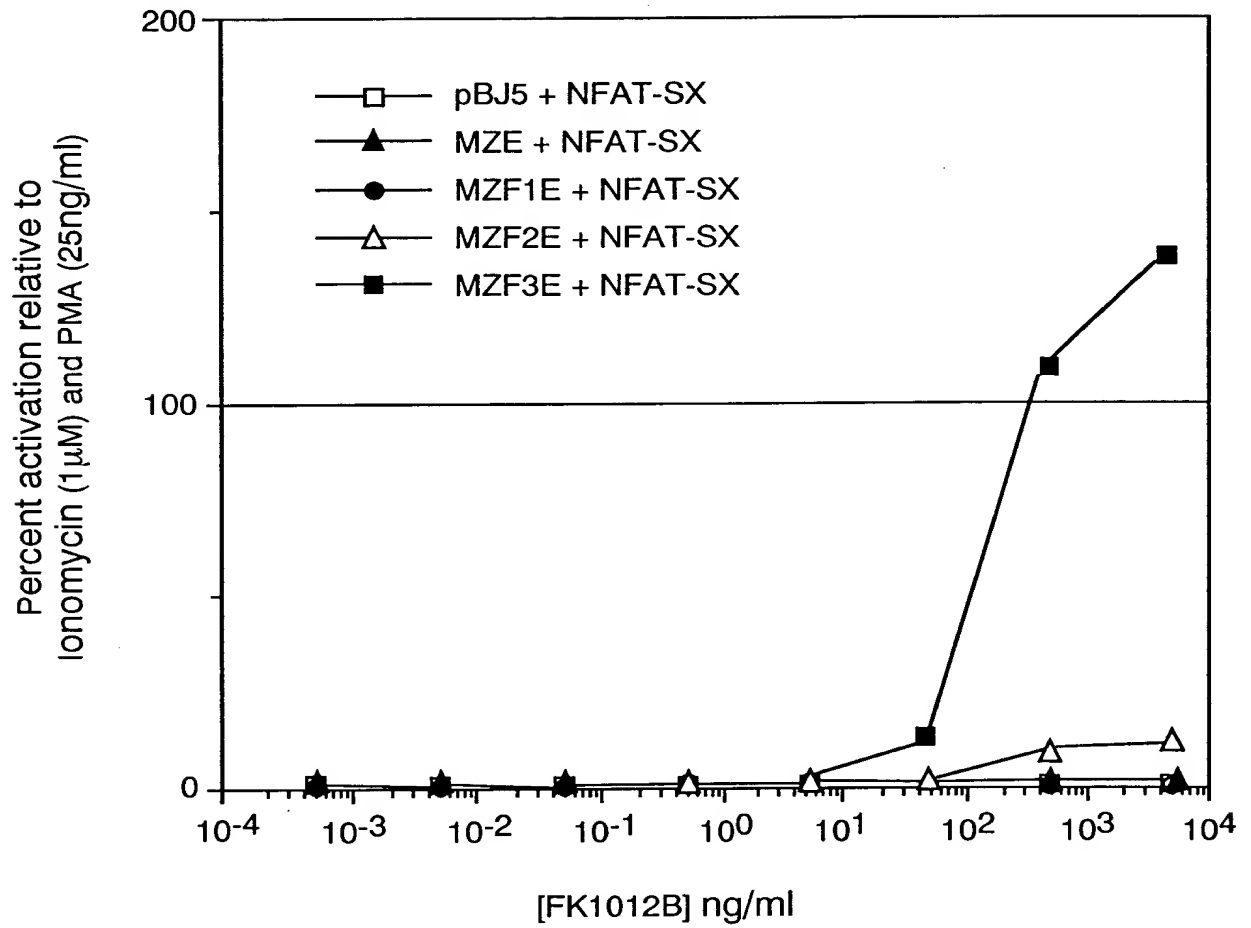


FIG. 8

000000000000000000

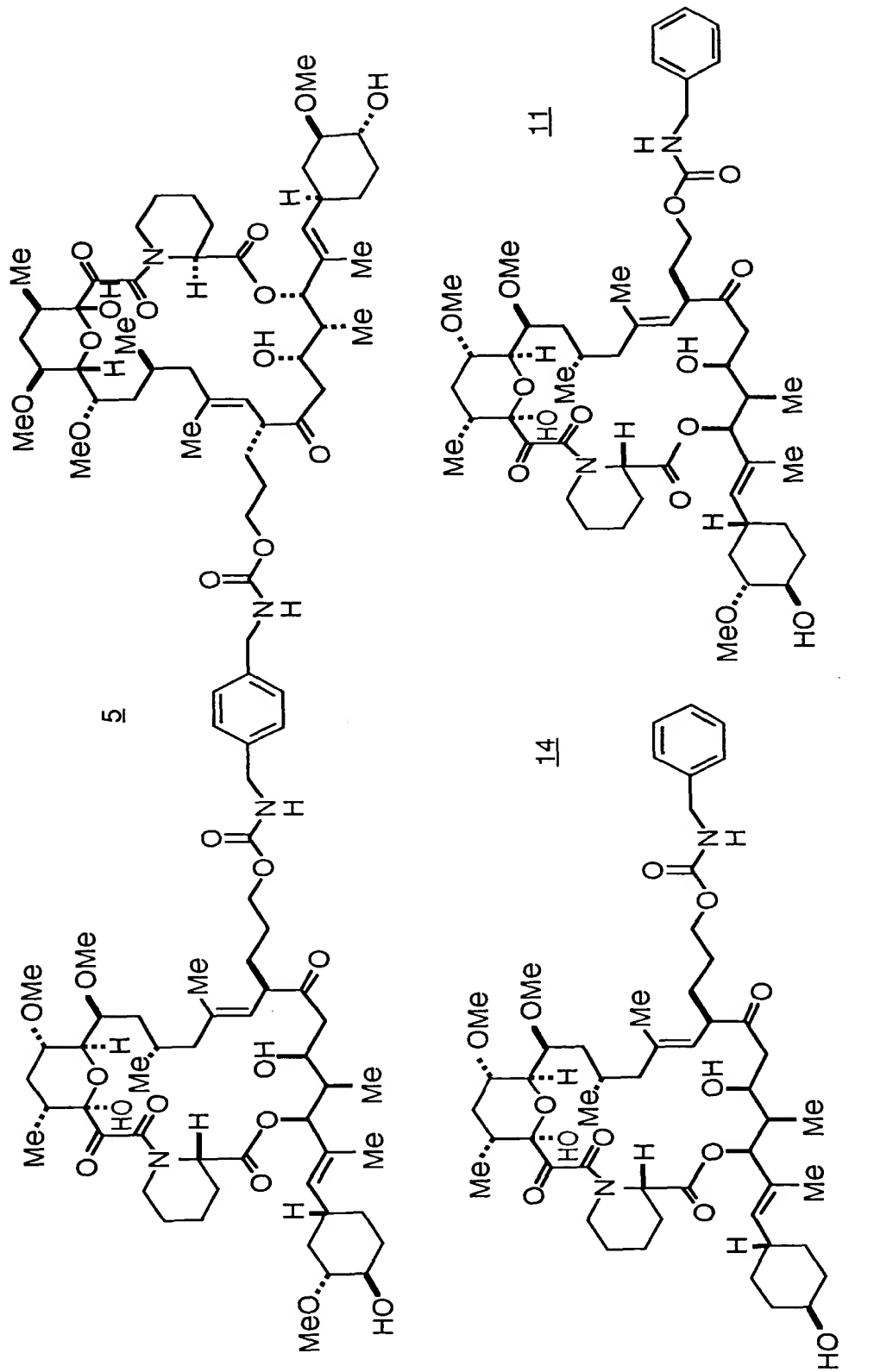
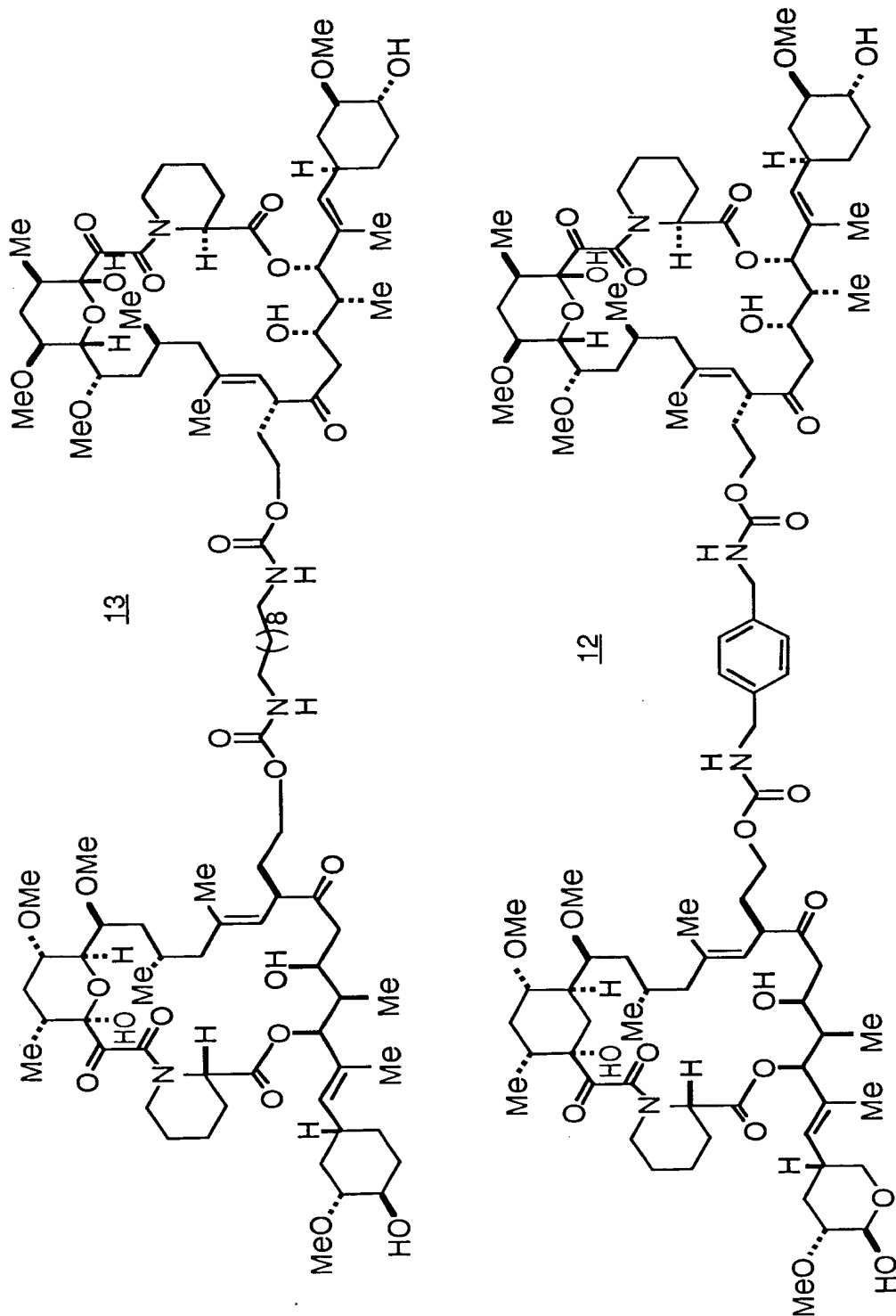
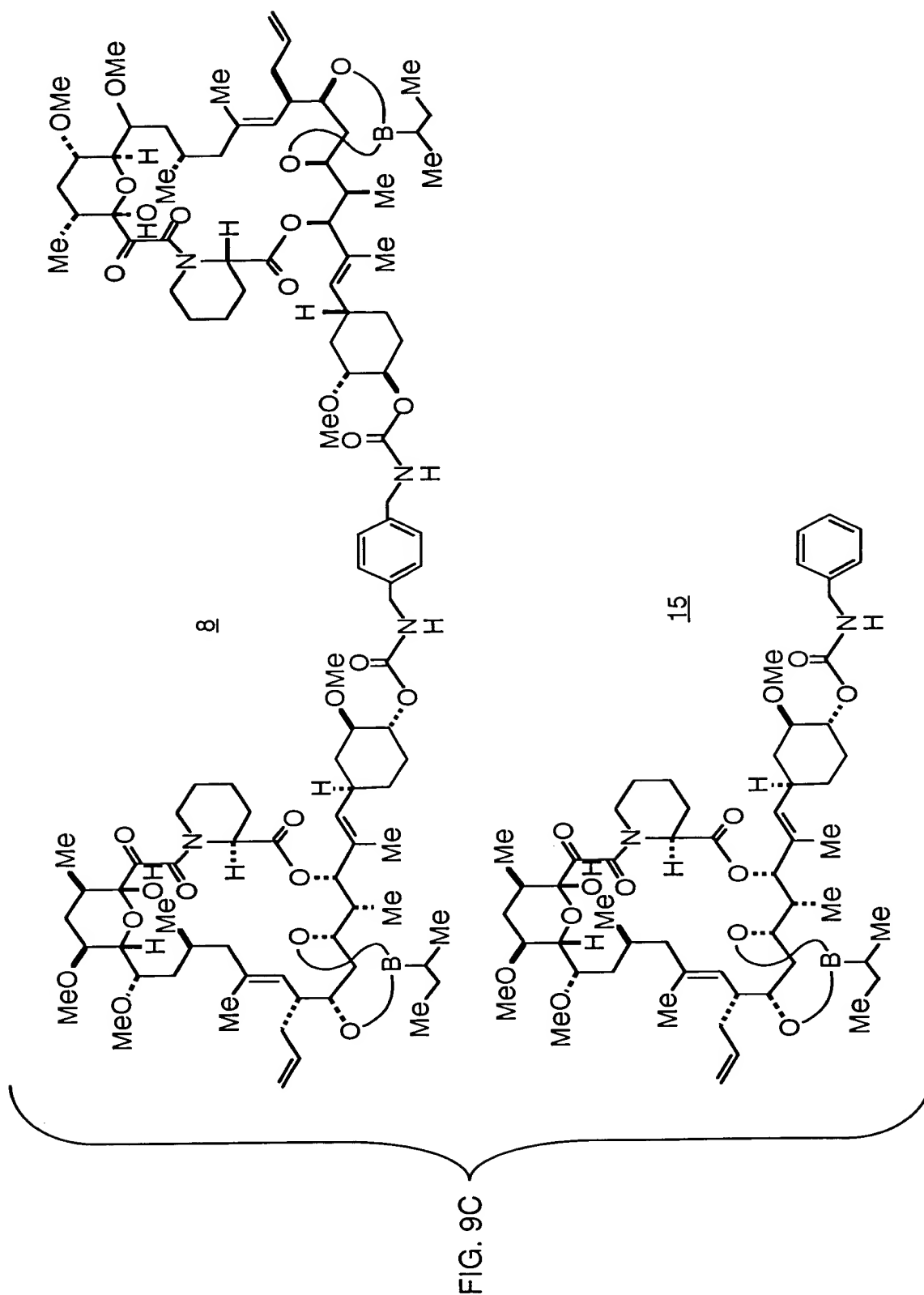
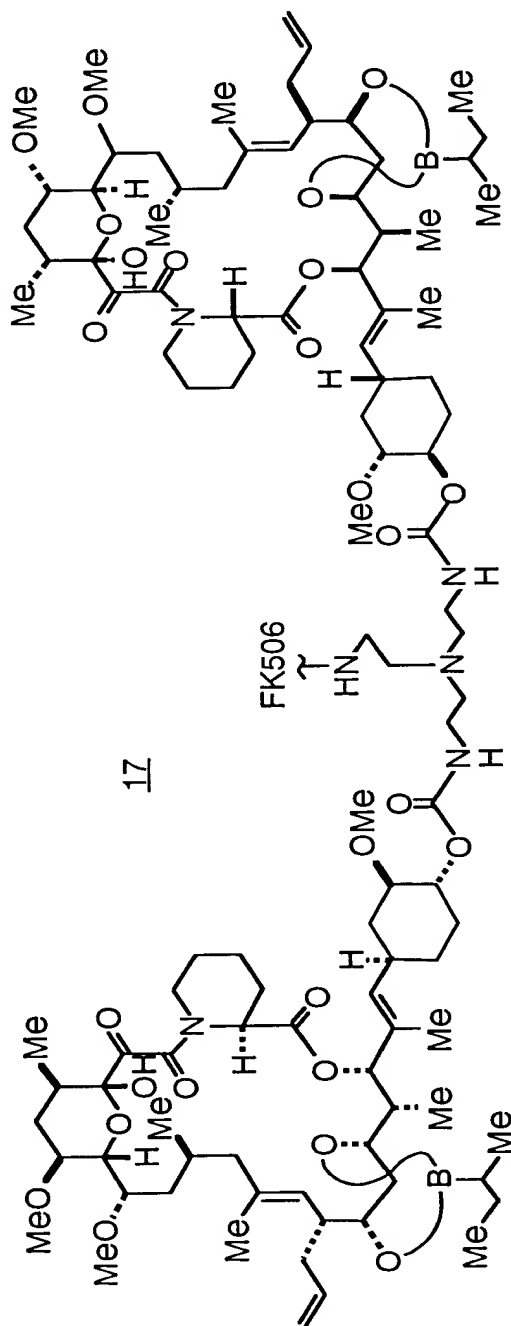
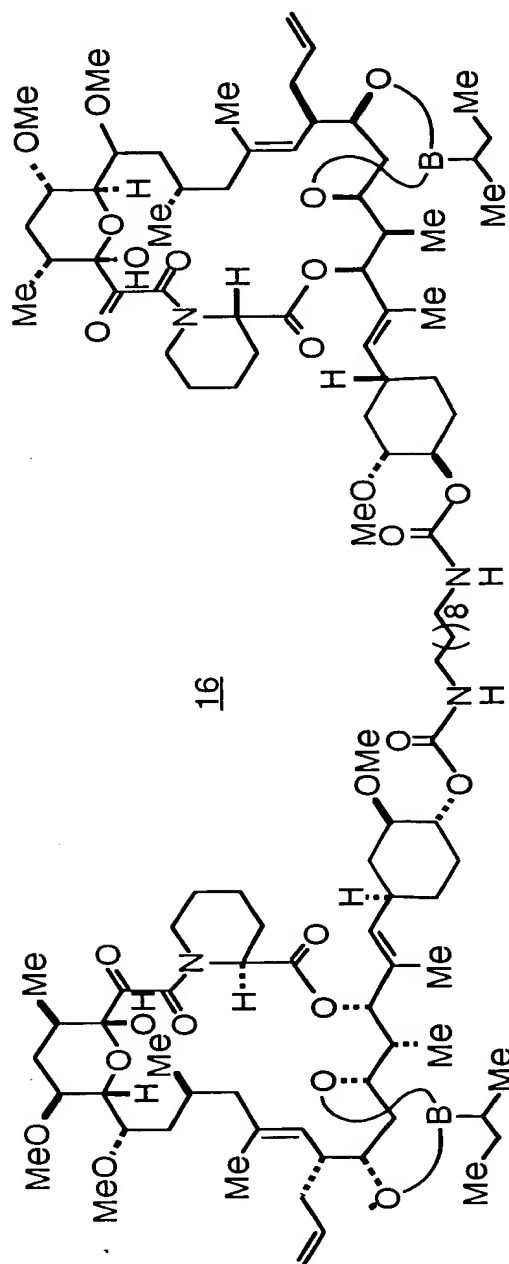


FIG. 9A

FIG. 9B







17/36

Scheme 1

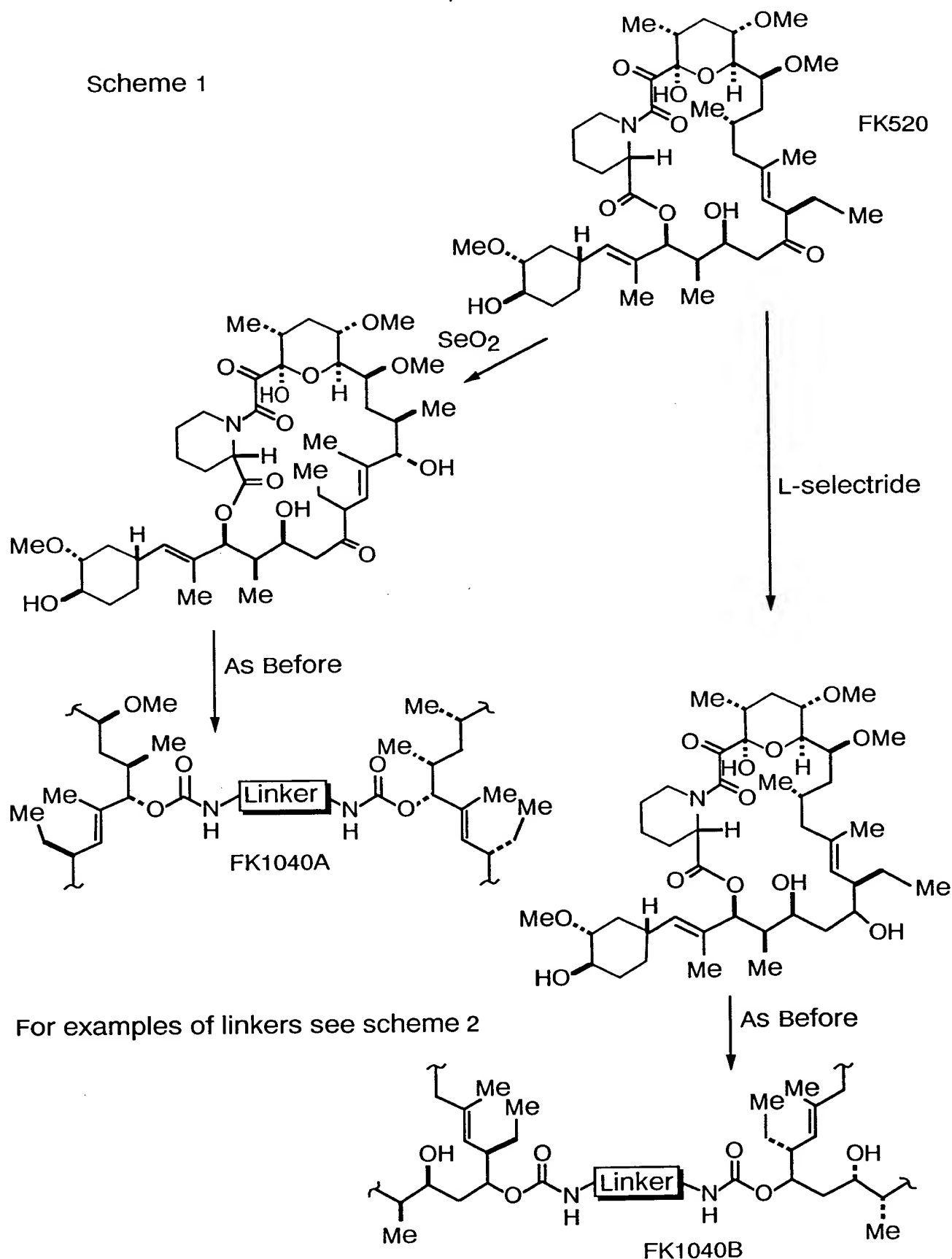
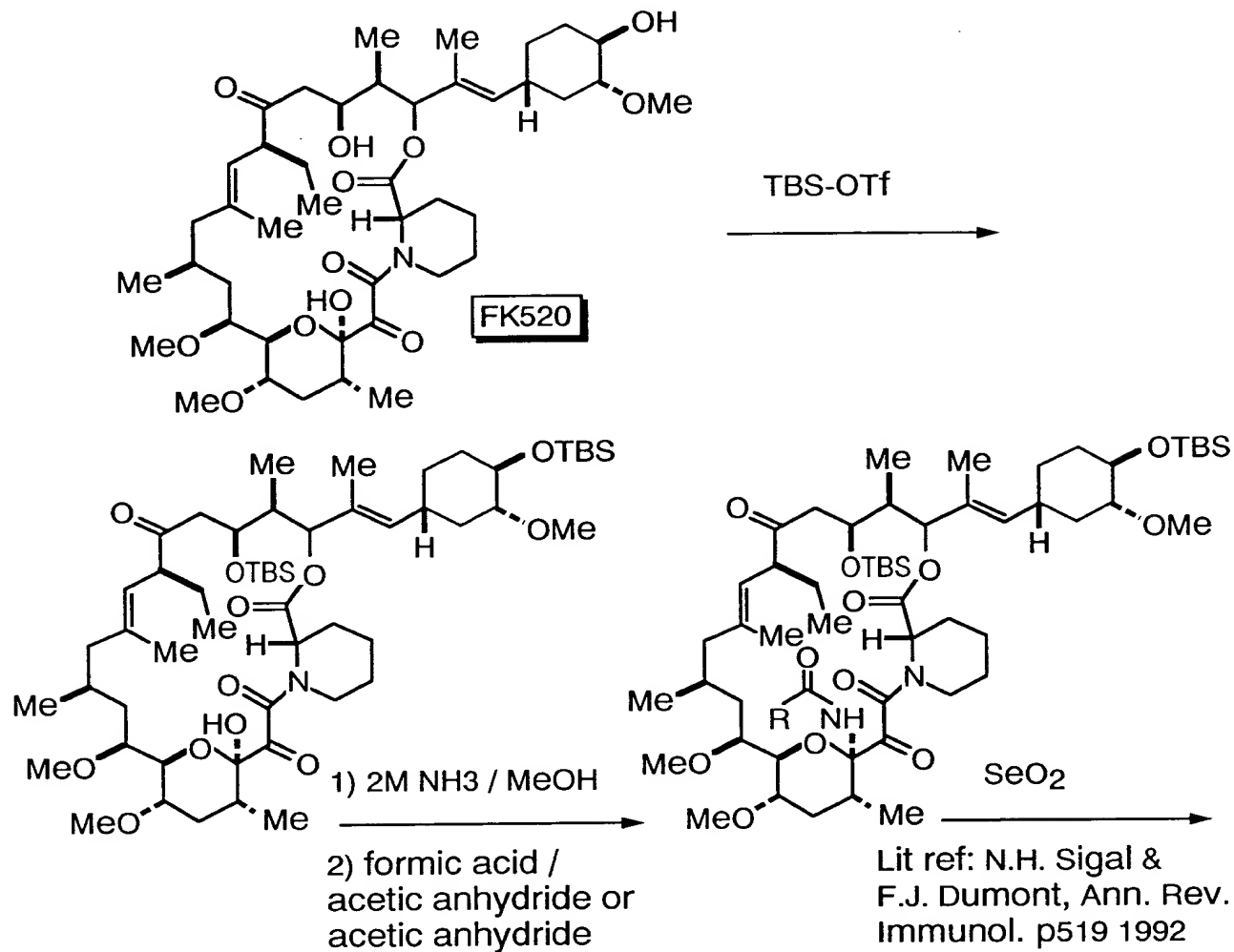


FIG. 10

Scheme 2: Synthesis of Dimers



Lit refs: D.K. Donald et.al. Tetrahedron Letters p1375, 1991, P.Kocovsky, Tetrahedron Letters p5521, 1992

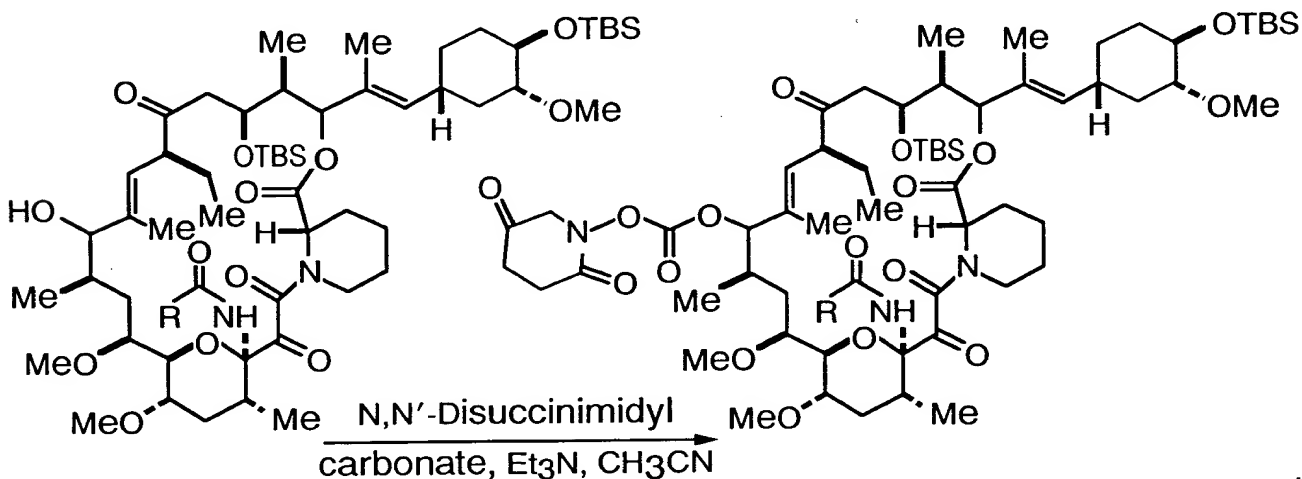


FIG. 11A

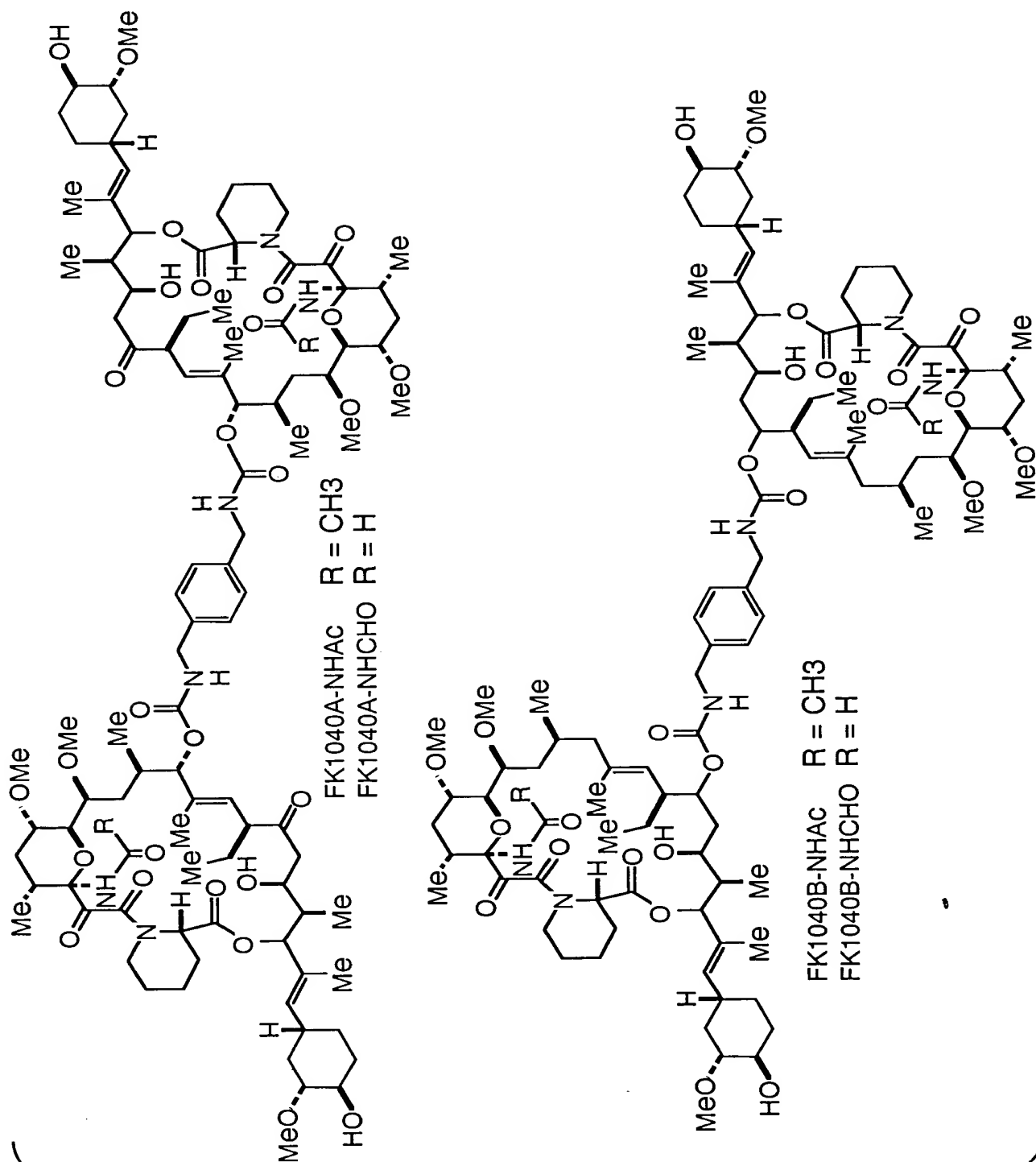


FIG. 11B

APPROVED	O.G. FIG.
BY	CLASS SUBCLASS
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20/36

An additional modified FK520 (FK1040) that interferes with FKBP12 yet should bind the FKBP12 mutant: F36A or F99A or Y26A, or combinations thereof is

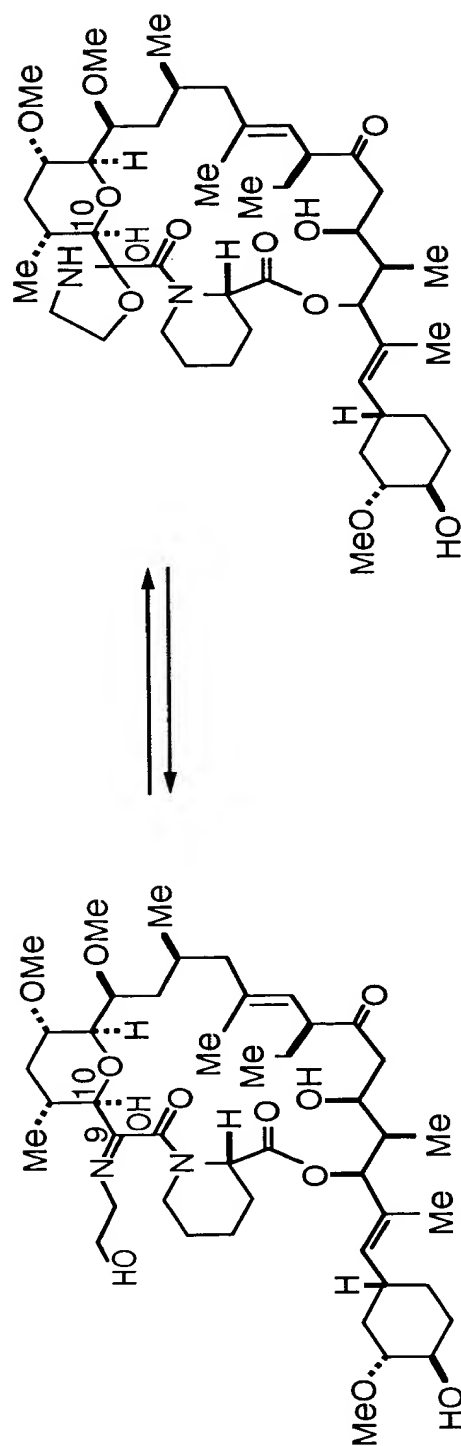


FIG. 11C

Scheme 3 Heterodimerization

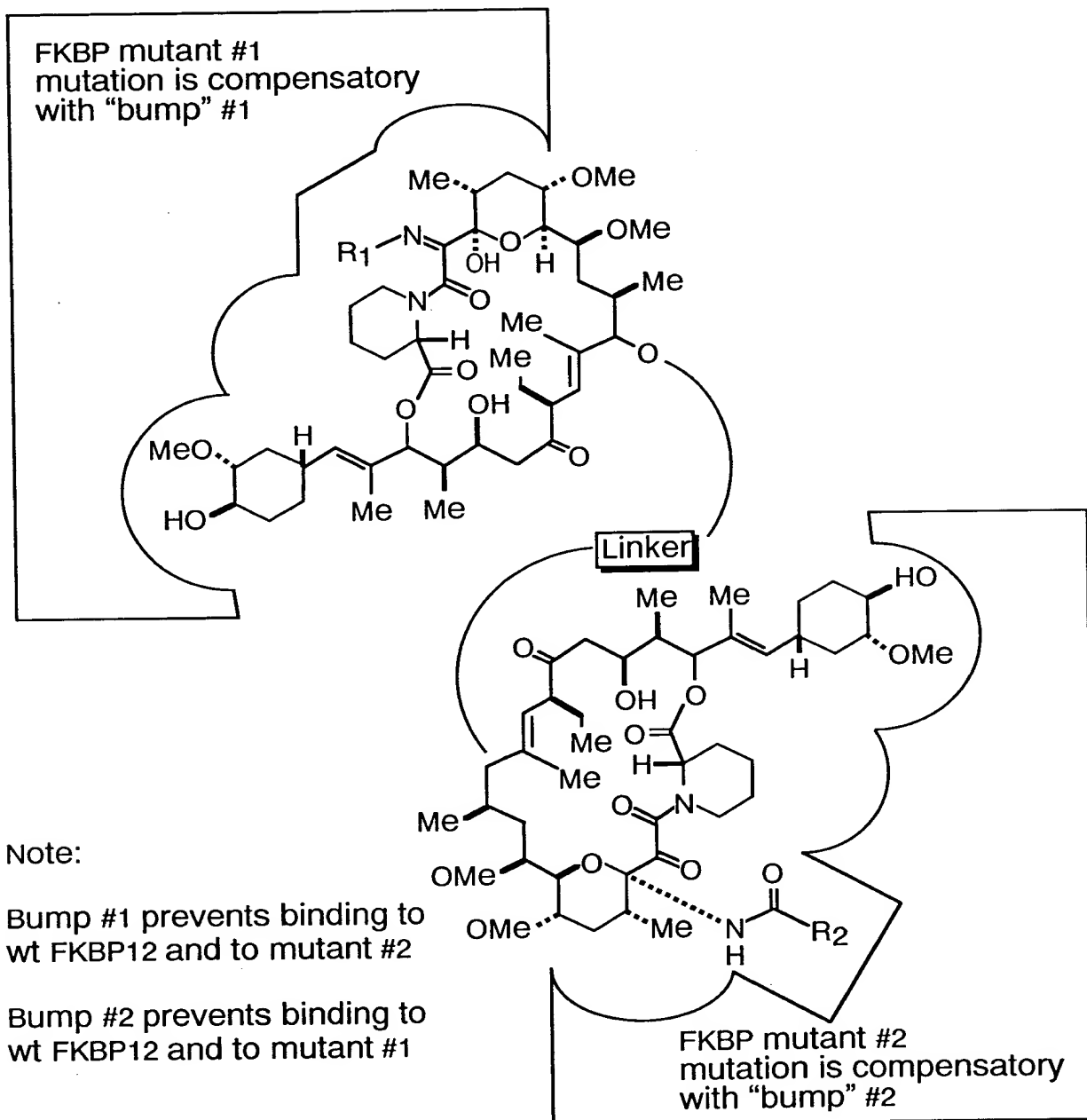


FIG. 12

22/36

Scheme 3: Synthesis of heterodimers

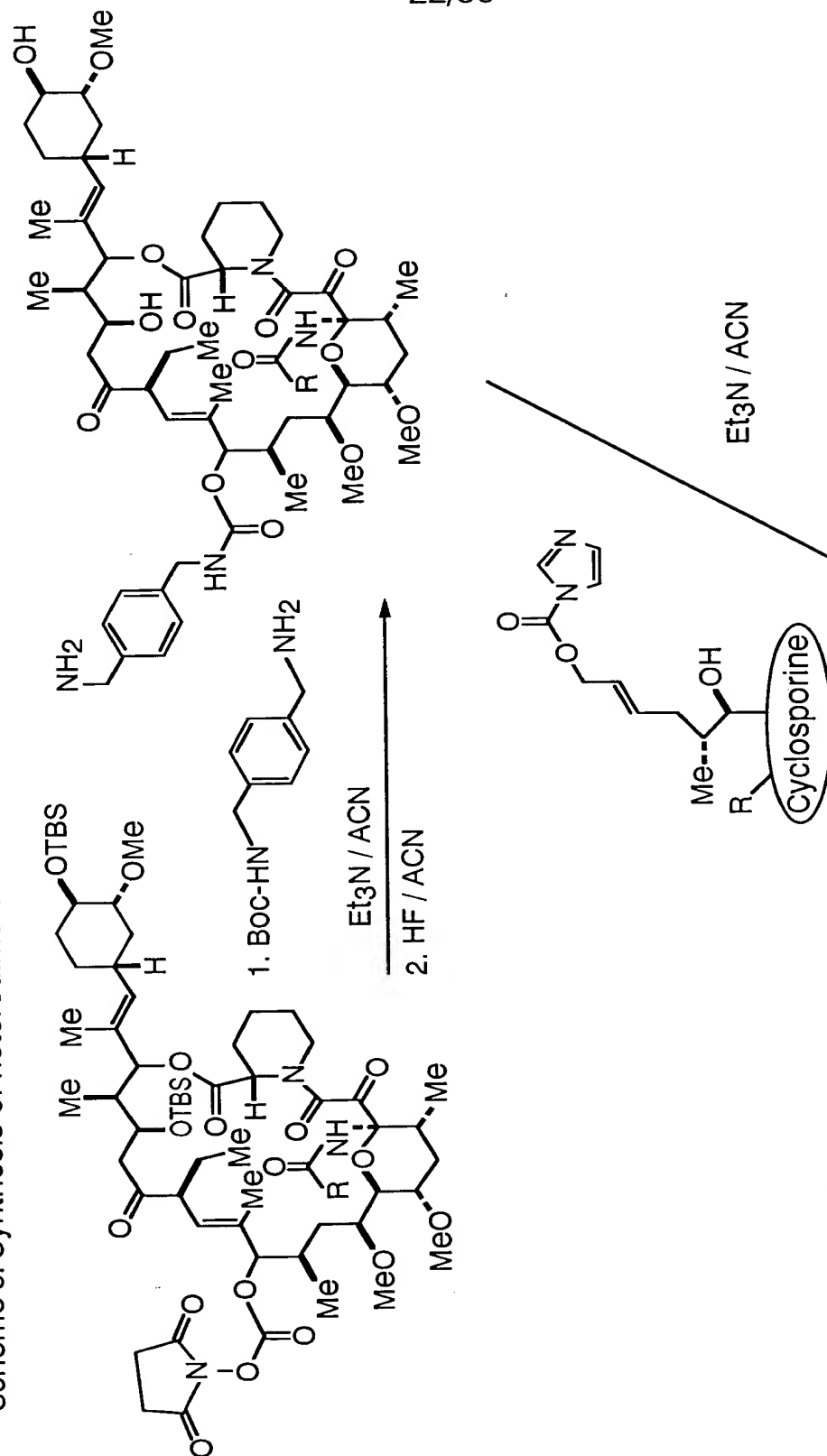
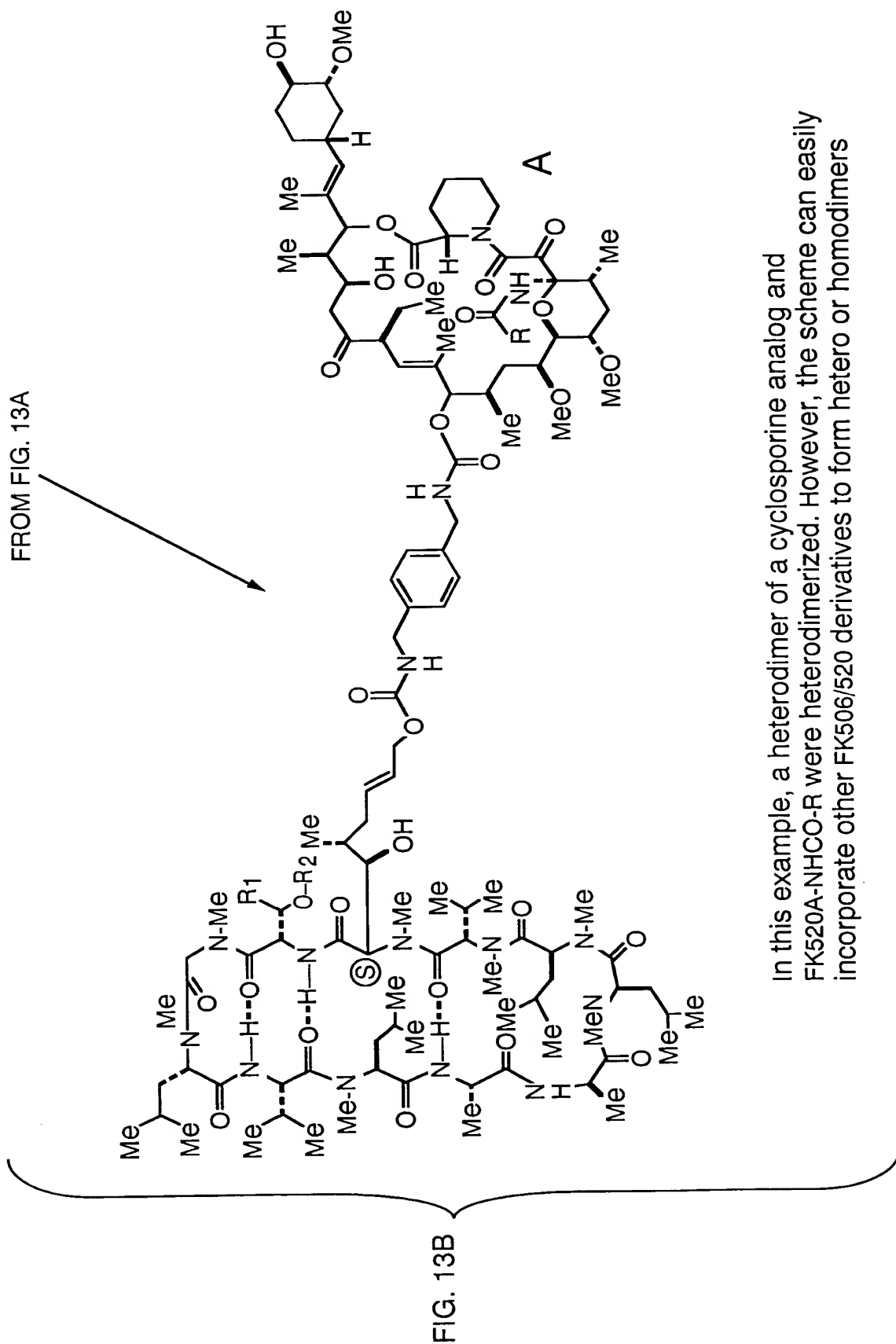


FIG. 13A



In this example, a heterodimer of a cyclosporine analog and FK520A-NHCO-R were heterodimerized. However, the scheme can easily incorporate other FK506/520 derivatives to form hetero or homodimers

SECRET

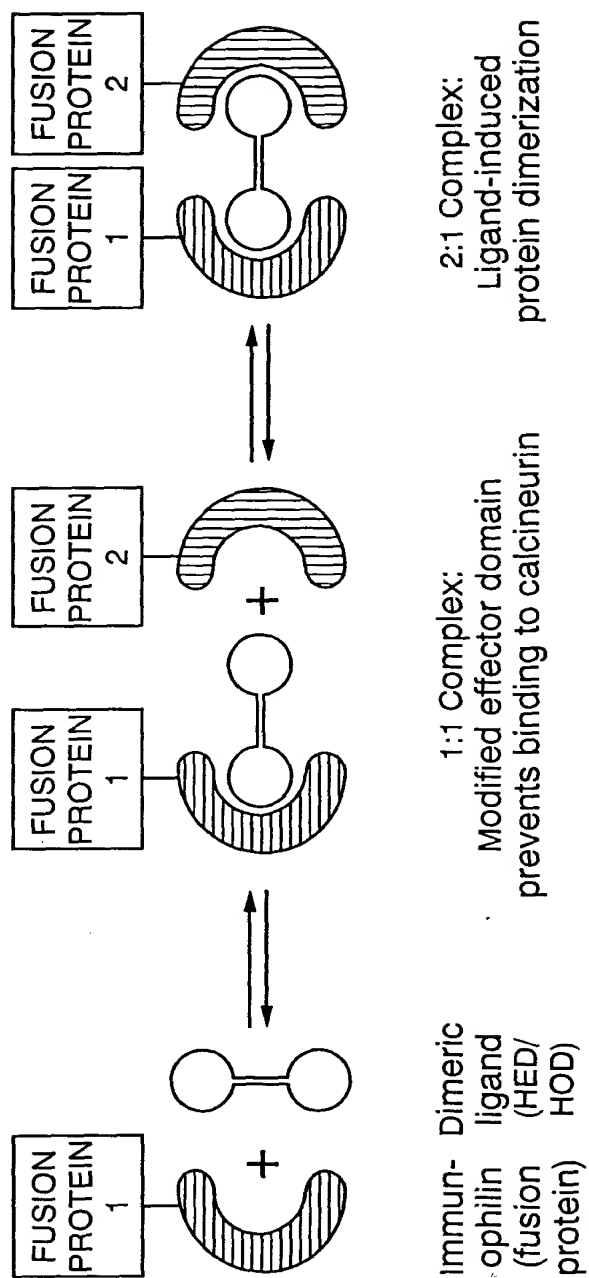


FIG. 14

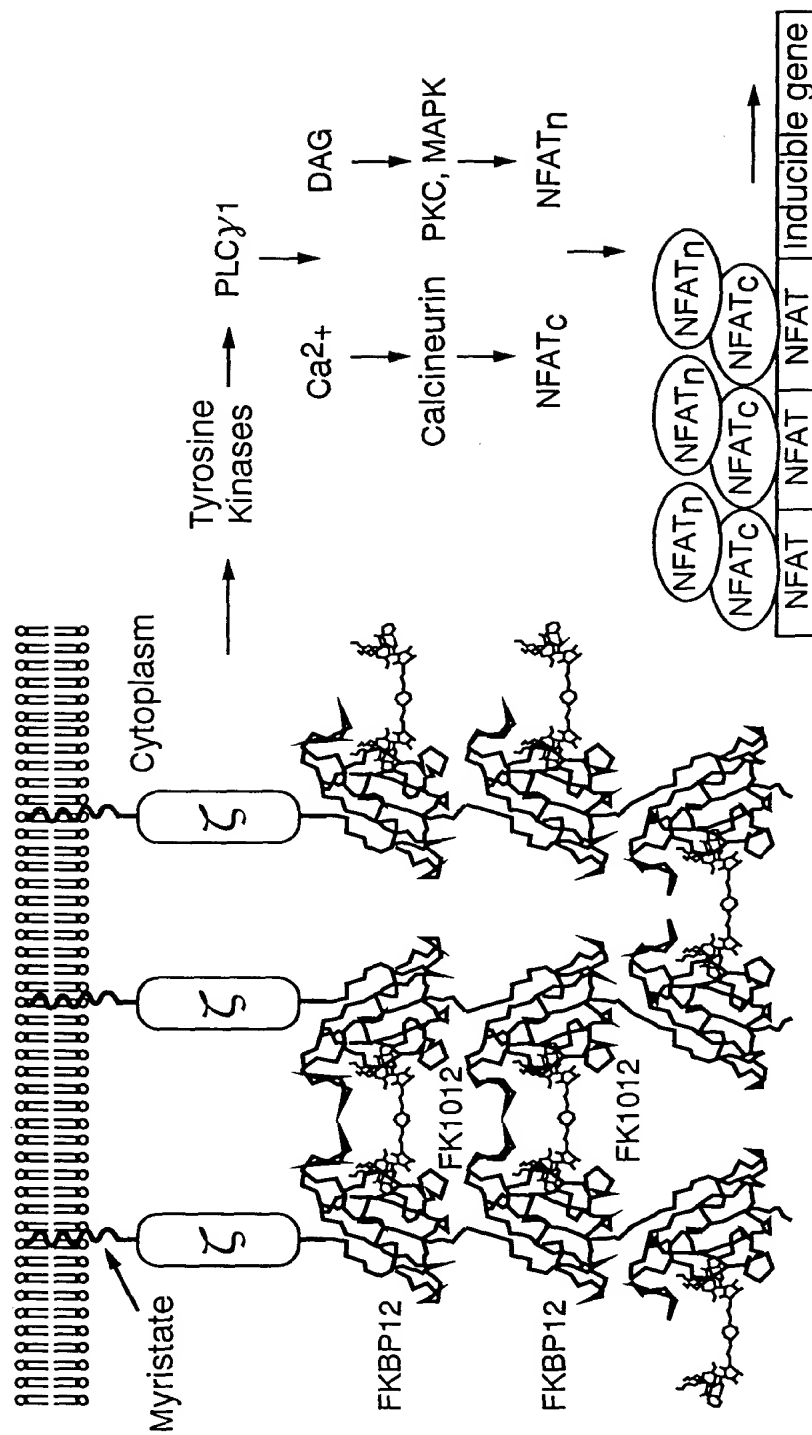


FIG. 15

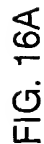
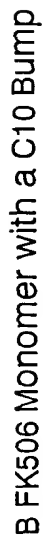
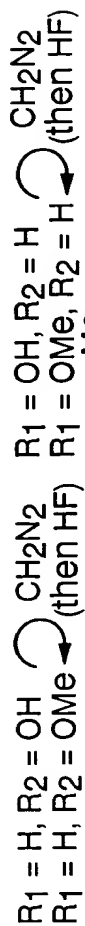
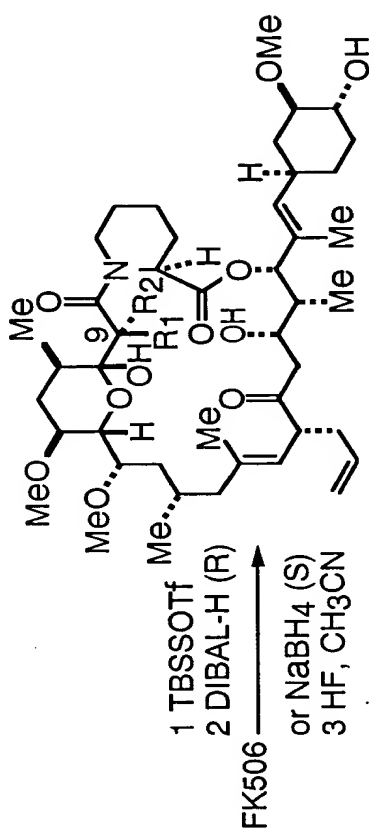
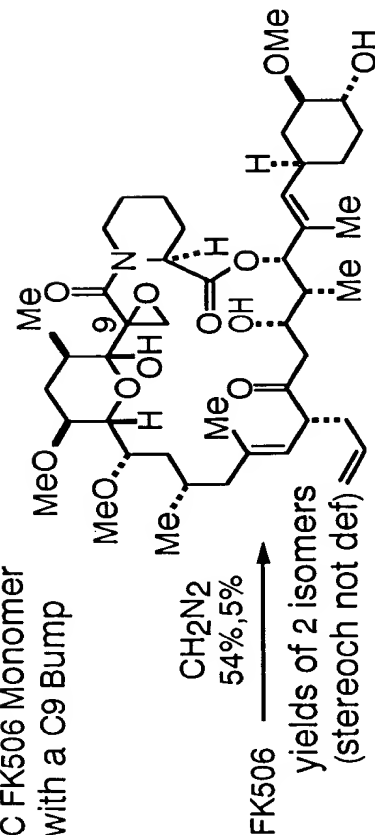


FIG. 16A



D HED Reagent Synthesis

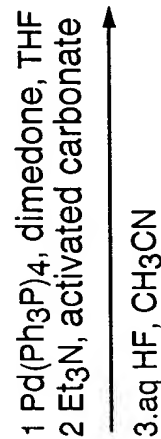
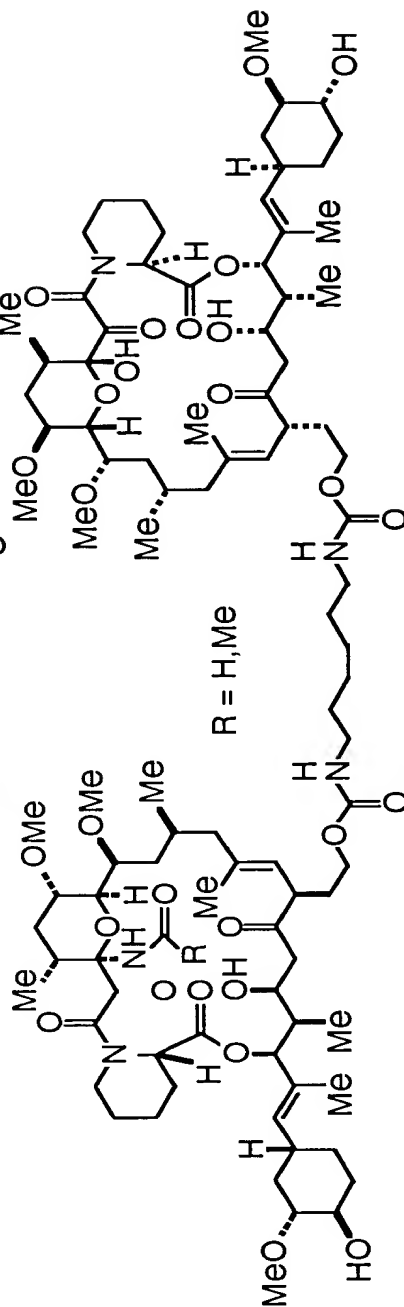
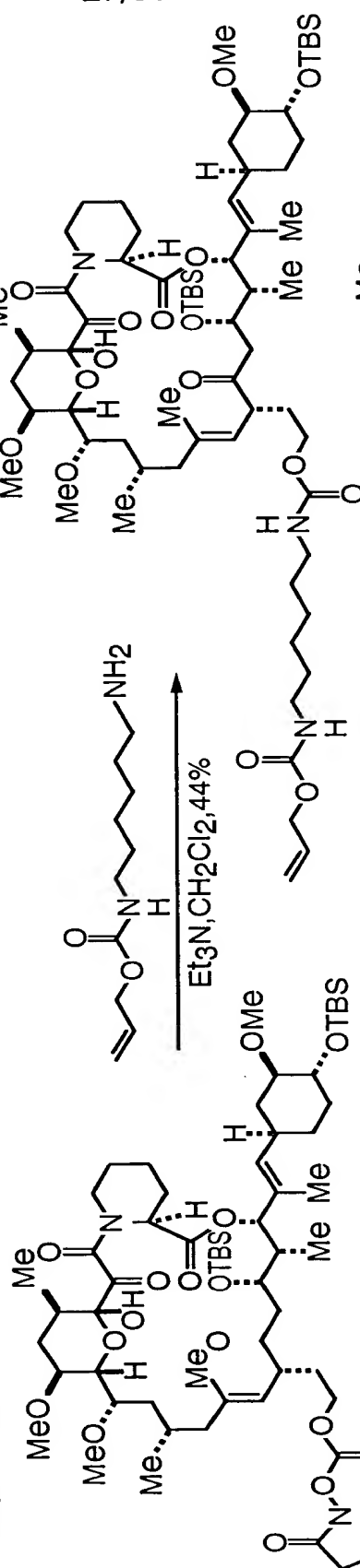
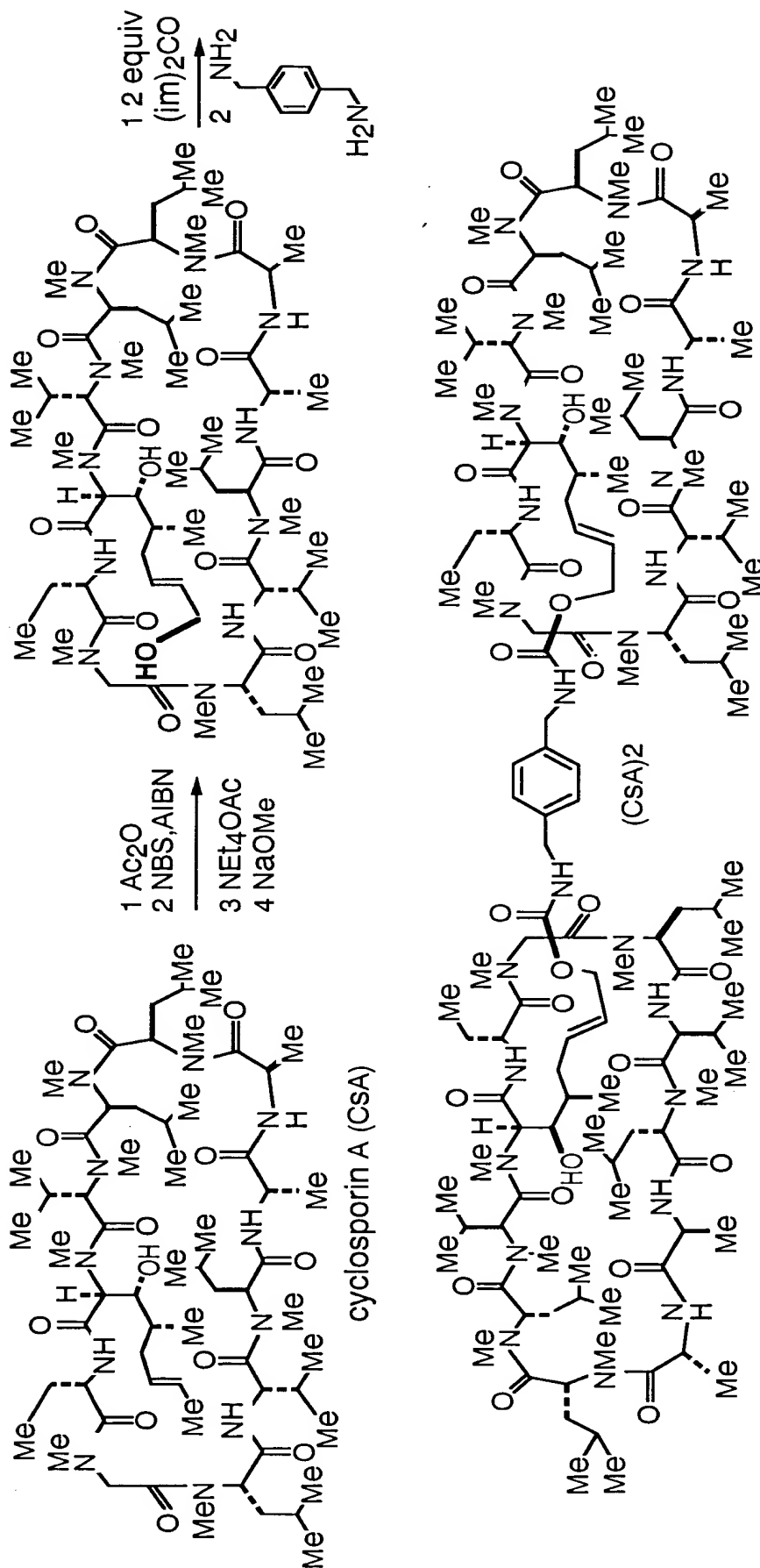
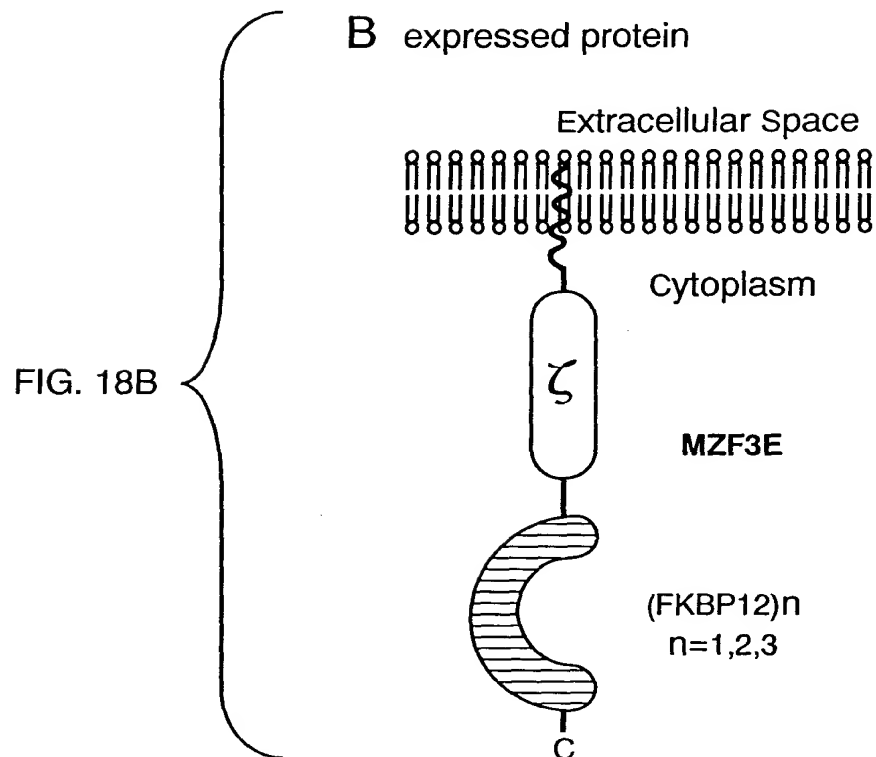
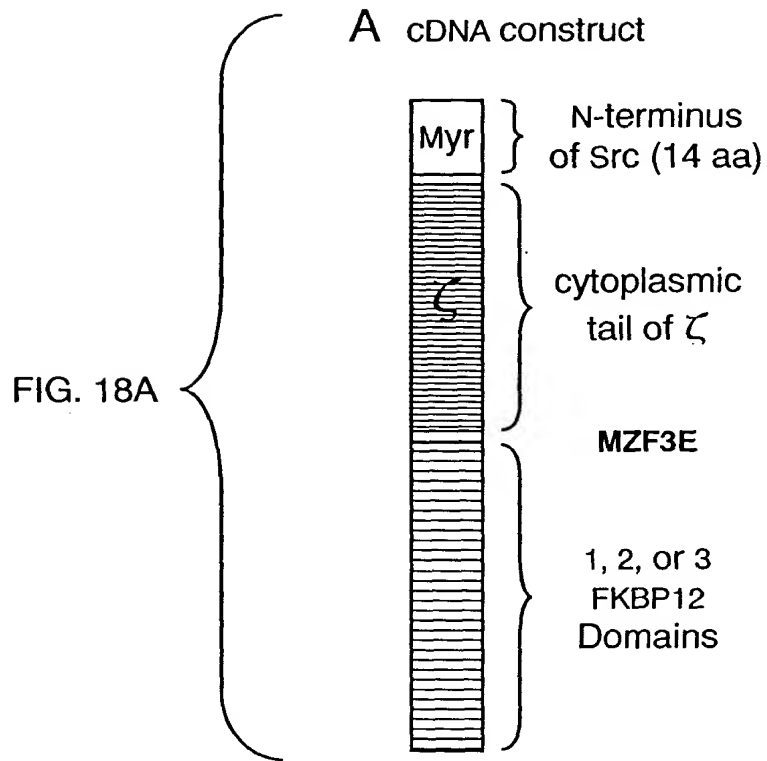


FIG. 16B





+

30/36

FK1012

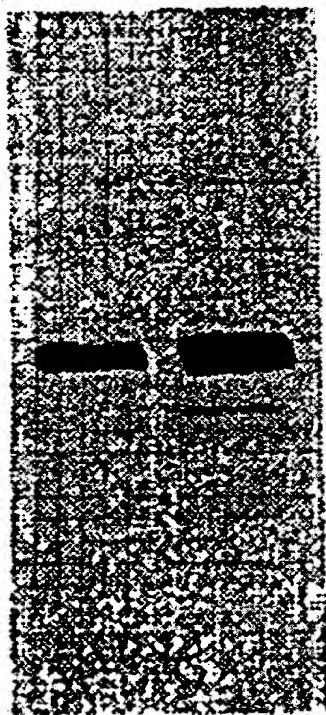
-

+

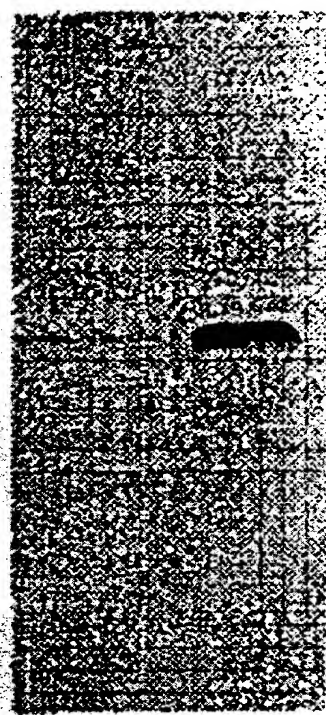
-

+

MZF1E_{f,h} -
(28kDa)



α Flag-blot



α Fiu-blot

FIG. 19



FIG. 20

[illegible]

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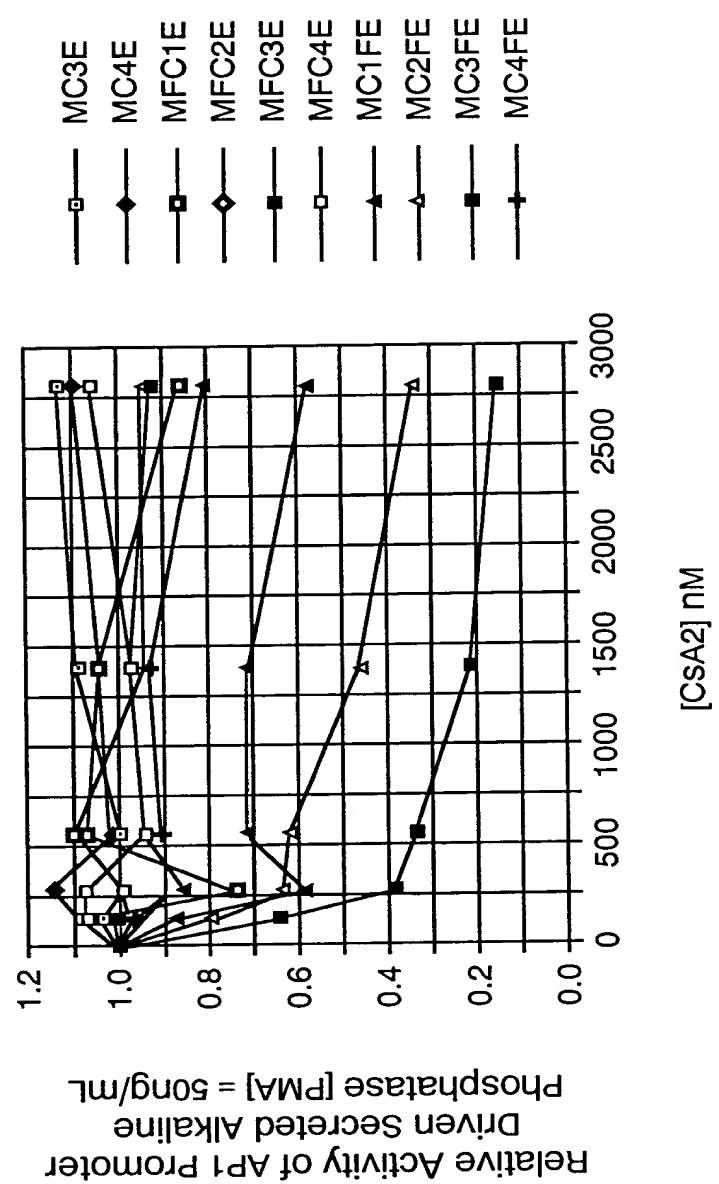


FIG. 21A

		LD50 Jurkat Cells				Relative Protein Expression
		Myr	Fas	FKBP	FKBP Ep	
A	MFF3E	Myr	Fas	FKBP	FKBP Ep	+
15nM						
B	MFC1E	Myr	Fas	CypC	Ep	-
	MFC2E	Myr	Fas	CypC	Ep	-
	MFC3E	Myr	Fas	CypC	CypC Ep	-
	MFC4E	Myr	Fas	CypC	CypC Ep	-
	MC1FE	Myr	CypC	Fas	Ep	+
	MC2FE	Myr	CypC	CypC	Fas Ep	+
	MC3FE	Myr	CypC	CypC	Fas Ep	+
	MC4FE	Myr	CypC	CypC	CypC Fas Ep	+/-
	MC3E	Myr	CypC	CypC	CypC Ep	+++
	MC4E	Myr	CypC	CypC	CypC Ep	++++
>30 uM						
>30 uM						

FIG. 21B

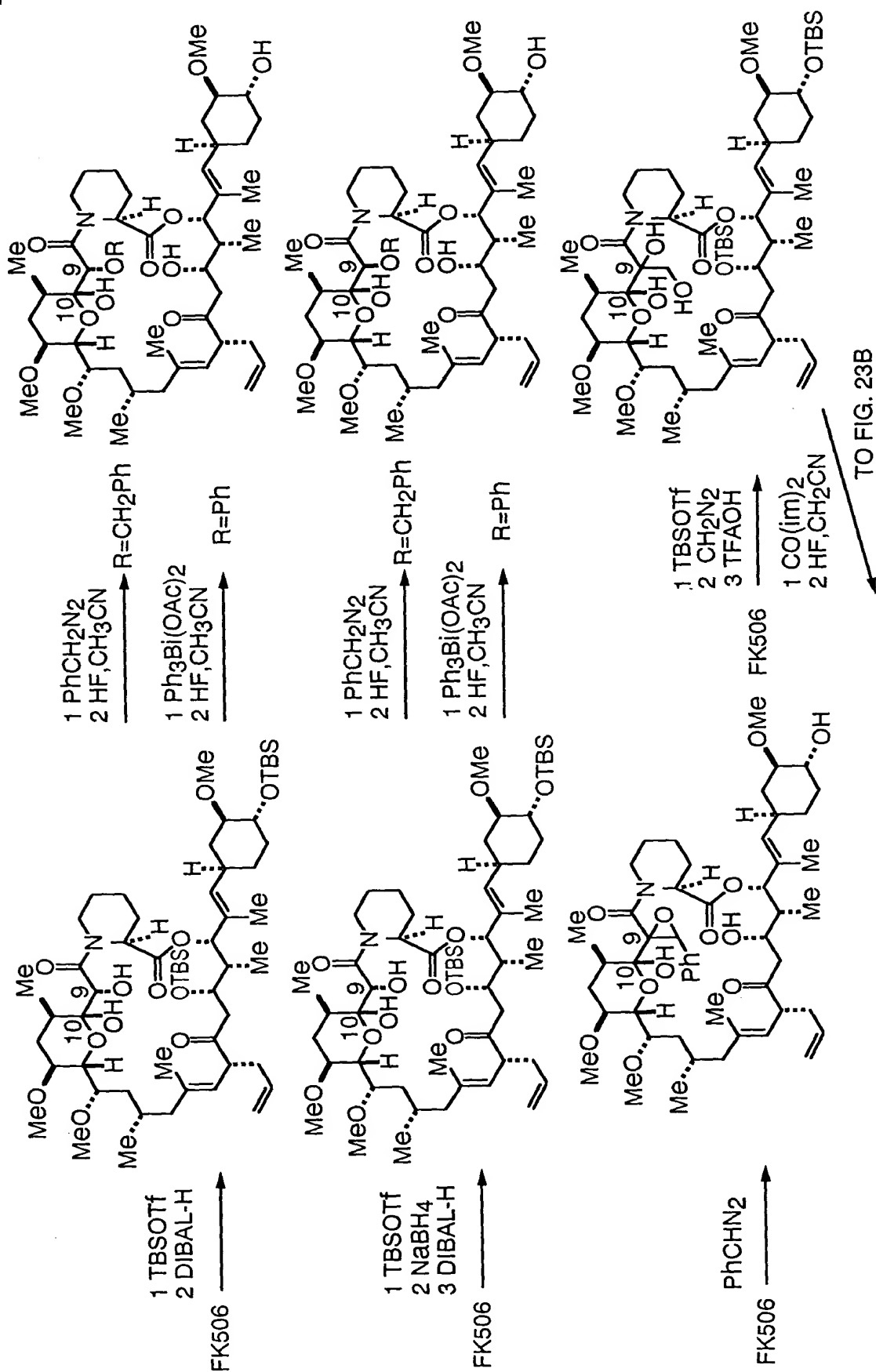


FIG. 23A

002127-095337160

